EXTENDED DATA FIGURE LEGENDS

Extended Data Figure 1. Performance of R5 reporter in T cell cultures. a-b, Flow cytometry with intracellular staining for IL-4, IL-13, IL-5 and IFN-γ of CD4+ T cells from wild-type and R5/R5 mice cultured under Th2 (**a**) or Th1 (**b**) conditions and then restimulated for 24 hours. Numbers represent percent of CD3+CD4+ cells. **c**, Percent of cultured CD3+CD4+ cells in the R5+ gate and ELISA for IL-5 from the supernatants in R5/+ and wild-type Th2 cultures restimulated on plate-bound anti-CD3ε for 4 days. Data are representative of two independent experiments (**a-b**), and ELISA data (**c**) obtained by averaging four replicates per timepoint.

Extended Data Figure 2. Surface markers and R5 expression in resting ILC2. a, Gating of cells from lung and small intestine. Numbers in first two panels (lung) and first panel (intestine) are percent of live (DAPI-) cells, and remaining panel previously gated on R5+CD90.2+ cells (lung) or R5+CD45+ cells (intestine). Histograms show R5+CD4-CD5- cells, total CD4+CD5+ cells, and eosinophils (SiglecF+CD11b+SSC hi). **b**, Percent R5+ of Lin-CD127+T1/ST2+ cells and R5 fluorescence in indicated tissues. **c**, IL-5 ELISPOT of R5+ cells sorted from the lungs R5/+ or R5/R5 mice and cultured

(3,000 cells/well) for 48 hours. Data are representative of two independent experiments with three mice per group (**a-b**), or representative of two independent experiments using sorted cells pooled from four mice (**c**). Represented as mean +/- SEM. Lin, Lineage markers (B220, CD5, CD11b, CD11c, Ly6G, FcεRI, and NK1.1); MFI, mean fluorescence intensity; *, $p < 0.05$; ***, $p < 0.001$ by Student's t test.

Extended Data Figure 3. R5+ ILC2 require IL7 and appear and persist after birth. a, Flow cytometry of lung cells from mouse strains as indicated. Numbers are percent of Lin- cells. **b**, Flow cytometry of lung cells previously gated as in (**a**) (Lin-CD90.2+T1/ST2+). Numbers are percent of Lin-CD90.2+T1/ST2+ cells. **c**, Total lung ILC2 (Lin-CD90.2+T1/ST2+) in mouse strains as indicated. **d**, Flow cytometry of R5/+ lung; previously gated on Lin-CD90.2+ cells. **e**, Lung Lin-CD90.2+T1/ST2+ cells as a percent of CD45+ cells at neonatal day 1, day 8, or week 8 of life. **f**, Percent BrdU+ of thymus CD4-CD8- (DN) cells, lung CD4+ T cells, and lung R5+ ILC2 after 14 days BrdU. Data are representative of two independent experiments (**a-b**, and **d**); pooled from three independent experiments for 4 (wild-type and IL7rα-/-) or 7 (all others) mice per group (**c**); pooled from three independent experiments for 5 (Day 1), 6 (Day 8), or 4 (Adult) mice per group (**e**); or pooled from two independent experiments for 3 mice per group (**f**). Represented as mean +/- SEM. Lin, Lineage markers (B220, CD5, CD11b, CD11c, Ly6G, FcεRI, and NK1.1); NS, not significant by Kruskal Wallis (**c**) or by Student's t test (**f**); *, $p < 0.05$; ***, $p < 0.001$ by Student's t test.

Extended Data Figure 4. Cre-mediated tracking or deletion of R5+ cells. a-b, Flow cytometry of R5/R5 and R5/R5 ROSA-YFP lungs twelve days after infection with *N. brasiliensis*, previously gated on live (DAPI-) cells. **a**, Numbers are percent YFP+ of live (DAPI-) cells (left two panels), and percent R5+CD4+ and R5+CD4- of YFP+ cells (second from right). Far right panel, CD90.2 and T1/ST2 staining of R5+CD4- cells. **b**, Numbers are percent of CD4+ cells. **c**, Baseline total cells and percent CD4+ T cells in bone marrow (single femur), spleen, and lung, and CD4+ cells as a percent of CD45+ cells in small intestine lamina propria of R5/R5 or R5/R5 Deleter mice. **d**, Representative flow cytometry of small intestine lamina propria cells (previously gated as CD45+CD8-NK1.1-). Data representative of 2 mice in each group from one experiment (**a-b**); or pooled from three independent experiments for 6 (small intestine, and R5/R5 bone marrow and spleen) or 9 (all others) mice per group (**c**); or representative of two mice in each group from one experiment (**d**). Represented as means +/- SEM. BM, bone marrow; ***, $p < 0.001$ by Student's t test.

Extended Data Figure 5. Activation by IL-2 and IL-33. a, Flow cytometry and quantification of R5 and KLRG1 fluorescence in ILC2 from untreated Rag1-/- and R5/R5 Rag1-/- mice, and R5/R5 Rag1-/- mice treated with IL-2 and IL-33. Lung cells previously gated as Lin-CD90.2+. Data pooled from two independent experiments for 3 (R5/R5 + PBS) or 8 (R5/R5 + IL-2/IL-33) mice per group. Represented as means +/- SEM. MFI, mean fluorescence intensity.

Extended Data Figure 6. Feeding enhances S13 expression. a, Schematic for feeding during light-only or dark-only 9 days prior to harvesting tissues. **b**, Flow cytometry of small intestine cells previously gated on CD45+Lin-CD127+ (left panels), or CD45+Lin-CD127-ICOS+R5+ (right panels) showing huCD4 and R5 fluorescence. **c**, Percent R5+ and R5 fluorescence of lamina propria ILC2 after 10-day food schedule in (**a**). **d**, Percent S13+ of lamina propria after 16-hour fast. Data representative of 2 independent experiments with 4 mice per group (**b-c**), or one experiment with 4 (fed) or 2 (fasted) mice per group (**d**). Represented as mean +/- SEM. Lin, Lineage markers (CD4, CD5, CD8, B220, CD11b, CD11c, NK1.1, Gr1); MFI, mean fluorescence intensity.

Extended Data Figure 7. Sorted ILC2 respond to VIP. a, Representative flow cytometry of ILC2 from small intestine of wild-type or R5/R5 mice, previously gated on Lin-CD45+KLRG1+. **b-c**, IL-5 in culture supernatant measured by cytometric bead array. **b**, Lin-CD45+KLRG1+ ILC2 sorted from small intestine cultured at 10,000/well in IL-7 (10 ng/ml) alone or with VIP (1 μM) for 6 hours. **c**, Lin-CD90.2+CD25+ ILC2 sorted from lung cultured at 5000/well in IL-7 (10 ng/ml) alone or with VIP or VPAC2-specific agonist BAY 55-9837 (both 1 μM) for 18 hours. Data are representative of three independent experiments (**a**) or pooled averages of duplicate cultures from 4 (**b**) or 3 (**c**) independent cell sorts. Lin, Lineage markers (CD4, CD5, CD8, B220, CD11b, CD11c, NK1.1, Gr1); *, $p < 0.05$ by paired Student's t test.